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Research Article
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Content of anthocyanins and flavonols in the fruits of *Cornus* spp.

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The genetic pool of the cornelian cherry (*Cornus mas* L.) at the M.M. Gryshko National Botanical Garden (NBG) of National Academy of Sciences of Ukraine includes more than 100 specimens collected from wild and cultivated plants in Ukraine, and cultivars of Bulgarian, Slovak, English, Austrian and Georgian selection. The genetic pool of cornelian cherry of the NBG presents a rich variety of biological and economic properties. Data on the content of anthocyanins and flavonols in the fruits of *Cornus mas* cultivars with early, medium and late fruit maturation periods are presented. The fruits of the *Cornus officinalis* Sieb. et Zucc. (CO-01, CO-02) and the hybrid *Cornus mas* × *Cornus officinalis* (cultivars Etude No 1 and No 2) were also studied. As a result of the study, it was found that the content of anthocyanins and flavonols in the fruits of *Cornus* has a significant difference in cultivars. Among *C. mas* cultivars with early fruit ripening, the most promising as a source of bioflavonoids preparations are 'Pervenets' and 'Volodimirskij', among *C. mas* cultivars with an average fruit ripening period, the most promising are the cultivars Mriya Shajdarovoi, Vydubetskyi and Titus, among the *C. mas* cultivars with a late fruit ripening period, the most promising are the cultivar Sokoline. Also promising are the hybrid *C. mas* × *C. officinalis* (cultivar Etude No. 1 and No. 2). These most promising cultivars and forms must commend for use in the food and medical sectors of the national economy.

Keywords: *Cornus mas*, *Cornus officinalis*, cultivar, fruits, anthocyanins, flavonols

Introduction

Subgenus *Cornus* L., which has a fragmented areal on the globe, is represented by four species: *Cornus mas* L. – in the west of the continent of Eurasia, *C. officinalis* Sieb. et Zucc. – in Japan, China and Korea, C. chinensis Wanger. – in the central region of China and *C. sessilis* Toor. – in North America (Browic, 1986).

C. mas (cornelian cherry) is a very ancient, cultivated plant, known in Ukraine as a culture since the time of Kievan Rus. The development of horticulture in Russia was associated with famous monasteries, especially Vydubetsky, Mezhyhirsky, Kyiv-Pechersk Lavra, it was here that many plants were introduced into culture, including cornelian cherry. The forms of these plants are very diverse, many of them can be directly introduced into the culture, and some can be converted into excellent cultivated plants by breeding. These plants include *C. mas* a very ancient fruit valuable food, medicinal, soil protection, ornamental plant, used in the Neolithic era. The modern range of cornelian cherry is the Pontic Mediterranean region: the southern Mediterranean regions of Europe, the southern foothills of the eastern Carpathians, as well as the Crimea, Caucasus and Asia Minor (Klymenko, 1990). Cornelian cherry is a culture that meets the standards of the time. According to the literature data and our research cornelian cherry yields are abundant and stable in culture (Klymenko, 2004; Klymenko et al., 2017b). The plant bares large juicy fruits, while not demanding thorough care. Its cultivation is very productive. Plants are usually not damaged by vermin and illnesses and do not need pest treatments.

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C. mas is recognized as a source of polyphenols, tannins, anthocyanins, and iridoids, all of which are present in both its fruits and leaves (Kucharska et al., 2015; Szczepaniak et al., 2019).

A high number of bioactive compounds have been identified in *C. mas* fruits (Klymenko et al., 2019), among which flavonoids exert favourable health effects especially by acting as potent antioxidants (Moldovan and David, 2017). Two iridoids (loganic acid and cornuside) and five anthocyanins (delphinidin, cyanidin, and pelargonidin glycosides) were identified in cornelian cherry fruits. The MS fragmentation pathways of the two iridoids were studied. The content of total iridoids in cornelian cherry fruits covered a wide range, from 86.91 to 493.69 mg/100 g fw. Loganic acid was the most dominant iridoid compound identified in cornelian cherry fruits and amounted to 88–96 % of total iridoids.

In most *C. mas* fruits, pelargonidin-3-galactopyranoside was dominant (Kucharskaa, et al., 2015). Cyanidin-3-rhamnosylgalactoside, a new anthocyanin, was isolated and identified from the berries of *C. mas*. Cyanidin-3-galactoside and delphinidin-3-galactoside were also identified (Du and Francis, 1973).

Bioactive compounds are present not only in flowers, but also in seeds, buds, shoots, leaves, pollen and bee pollen e.g. (Brindza et al., 2009; Krivoruchko etal., 2011; Hosseinpour-Jaghdani et al., 2017; Klymenko et al., 2017a; Levon et al., 2017; Brindza et al., 2018; Grygorieva et al., 2020; Przybylska et al., 2020).

The content of flavonoids in leaves of *C. mas* is 847.6 mg/100 g, in flowers – 1704.9 mg/100 g; in flowers of *C. officinalis* – 1448.9 mg/100 g (in terms of rutin). The content of acids in flowers of *C. mas* is 41.5 mg/100 g, in flowers of *C. officinalis* – 124.9 mg/100 g (in terms of chlorogenic acid). In leaves of *C. mas* rutin was identified, in flowers of *C. mas* and *C. officinalis* – chlorogenic and ellagic acids, rutin, kaempferol-3-O-glucoside and quercetin. The flavonoid rutin predominates in all studied samples (Krivoruchko, 2018). Based on HPLC–PDA–MS/MSn analysis eight compounds have been identified as quercetin, kaempferol, and aromadendrin glycosilated derivatives (Pawlowska, et al., 2010).

Juice, jam and compote from *C. mas* fruits are useful for anaemia, liver diseases, gout, stomach diseases (Sklyarevsky, 1975), Infusions of leaves and flowers are known as antipyretic and diuretic agents, and from the bark – as a tonic and stimulating (Chikov and Laptev, 1976). Medicines from fruits and leaves are used as astringents and disinfectants, selectively acting even on dysentery bacillus, typhoid pathogens. Cornelian cherry is also used for rheumatism, colds, fever and skin diseases (Blaze, 2000).

C. officinalis – native to Japan, Northeast China and Korea, is virtually unknown in Europe and grows only in the collections of Botanical Gardens. *C. officinalis* a relative of *C. mas*, native to northeastern China and Korea. It is cultivated in large areas in Japan – the local name – sandzaki. It is one of the important types of plant material used in Chinese traditional medicine. Is being quite common in Asia, and the UK, it is used even more widely than *C. mas*. In its homeland, *C. officinalis* is widely known as a medicinal plant. Studies conducted *in vitro* and *in vivo* have shown a multi-faceted protective effect of fruit extract against diabetes and its complications, in particular a diabetic nephropathy. Decoctions of fruits and leaves are used in folk medicine as a tonic, stimulating and astringent (Klymenko, 2002; Klymenko and Ilyinska, 2020).

Besides, we have researched cultivar Etude, which is an artificial hybrid from crossing *C. officinalis* × *C. mas*. Genotype obtained from grafting *C. officinalis* on *C. mas* (Klymenko and Ilyinska, 2020).

The presence of a large amount of biologically active substances in representatives of the Cornaceae family not only makes them a valuable medicinal raw material but also increases their resistance to many stress factors. Long-term observations have shown that representatives of the Cornaceae family have extremely high resistance to both pests and adverse weather conditions, plants bloom profusely and give birth (Klymenko, 2000).

All parts of plants of species of representatives of the Cornaceae family (fruits, leaves, bark, roots) are medicinal raw materials and have long been used in folk medicine for the treatment of anaemia, metabolic disorders, as an anti-scurvy, antidiabetic agent (Mamedov et al., 1990; Czerwińska and Melzig, 2018).

Very often medicinal cornel is used as an anti-diabetic medicinal plant, especially in East Asia. Fresh fruit treat diabetes, they reduce the level of glucose in the blood, increases the enzymatic activity of the pancreas, stimulate the processes of digestion. In Tibetan medicine, bark and leaves of medicinal cornel are used for the treatment of pleurisy, fever, powder of dried fruits to treat kidney illnesses (nephritis) (Yokozawa et al., 2009; Cao et al., 2011).

The purpose of this study was to determine the content of anthocyanins and flavonols in the fruits of plants of the genus *Cornus*. This will allow us to identify the most promising cultivars and forms for use in the food and medical sectors of the national economy.

Material and methodology

Biological material

Biochemical studies were carried out on fruits dried in a well-ventilated room in the absence of direct sunlight at a temperature of 20–25 °C and crushed in an electric mill. The fruits of *Cornus mas* plant cultivars were studied with early (Radist, Olena, Ekzotychnyi, Nespodivanyi, Elegantnyi, Nikolka, Nizhnyi, Pervenets, Volodymyrskyi, Samofertylnyi, Alyosha, Koralovyi Marka), intermediate (Koralovyi, Yantarnyi, Medok, Priorskyi, Yevgeniia, Svitliachok, Vyshgorodskyi, Lukianivskyi, Mriia Shaidarovoi, Starokyivskyi, Yoliko, Titus, Oryginalnyi, Kostia, Vydubetskyi) and late (Semen, Sokolyne, Yuvileinyi Klymenko, Kozerig, Kolisnyk), hybrid *Cornus mas* × *Cornus officinalis* (cultivars Etude No 1 and No 2) and two varieties of *C. officinalis* (CO-01 and CO-02). Plants collected from the collection of Department of Fruit Plants Acclimatization in M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) at the stage of fructification during 2019–2020. Biochemical analyses were carried out in the laboratory of Department of Fruit Plants Acclimatization of M.M. Gryshko National Botanical Garden.

Determination of total anthocyanins content

The quantity of anthocyanins was determined using a spectrophotometric method at a wavelength of 530 nm, using alcohol extraction from a homogenate of plant raw materials acidified with 3.5 % hydrochloric acid (Kriventsov, 1982).

The number of parallel determinations was 3. The accuracy of the method was in the range of 2.5–4.8 %. The data obtained were presented as mg/100 g dry matter (DM) in terms of cyanidin glycosides.

Determination of total flavonols content

To determine flavonols, an analytical sample of dried crushed fruits weighing 0.2–0.3 g was transferred to a flask, 3 ml of 80 % ethyl alcohol was added, and heated with a reflux coolbox for 45 minutes in a water bath. After that, the flask was cooled to room temperature and the suspension was filtered through a paper filter into a 100 ml volumetric flask. The resulting solution was brought to the mark with 80 % alcohol (solution A). 2 ml of solution A was placed in a 25 ml volumetric flask, 1 ml of a 2 % solution of aluminum chloride in 95 % ethanol was added, and the volume of the solution was brought to the mark with 95 % alcohol. The optical density of the solution was measured after 20 minutes at a wavelength of 390 nm in a cuvette with a layer thickness of 10 mm. The control was a mixture of solutions of aluminum chloride and acetic acid (Andreeva and Kalinkina, 2000).

The number of parallel measurements was 3. The accuracy of the method was in the range of 0.3–2.0 %. The obtained data were presented in mg/100 g DM.

The optical density of all the studied solutions was measured using a Zalimp KF 77 spectrophotometer (Poland).

Statistical analysis

Statistically processed data is shown on histograms as arithmetic means and their standard errors. The significance level was set at α = 0.05. The statistical analysis was performed with IBM SPSS Statistics, release 26.0.0.1.

Results and discussion

According to our previous data (Ma et al., 2014; Biaggi et al., 2018; Bayram and Ozturkcan, 2020; Demir et al., 2020), representatives of the genus *Cornus* are rich in biologically active compounds. A major role in the formation of medicinal properties of cornelian cherry is due to the action of the secondary metabolites of the class of flavonoids – anthocyanins and flavonols (Lila, 2004, Horbowicz et al., 2008), which are also considered as environmental markers of plants and participate in adaptive reactions of the plant organism (Minaeva, 1978; Lukner, 1979; Levon and Golubkova, 2016; Levon and Goncharovska, 2017).

Recently it was found that flavonoids affect signalise processes in living systems due to specific interactions with proteins, which perform regulatory functions (Stevenson and Hurst, 2007). Besides, flavonoids play an important role in the protection of plants against bacterial, viral and fungal infections, against the intrusion of vermin and insect damage. The biological role of flavonoids is their involvement in redox processes that occur in plants (Tarahovsky et al., 2013). Physical damage to plant tissues also leads to starting of the processes of oxidative degradation of flavonoids, preventing the development of wound infections (Walker, 1998).

The amount of anthocyanin in some cultivars of *C. mas* breeding of M.M. Gryshko National Botanical Garden of

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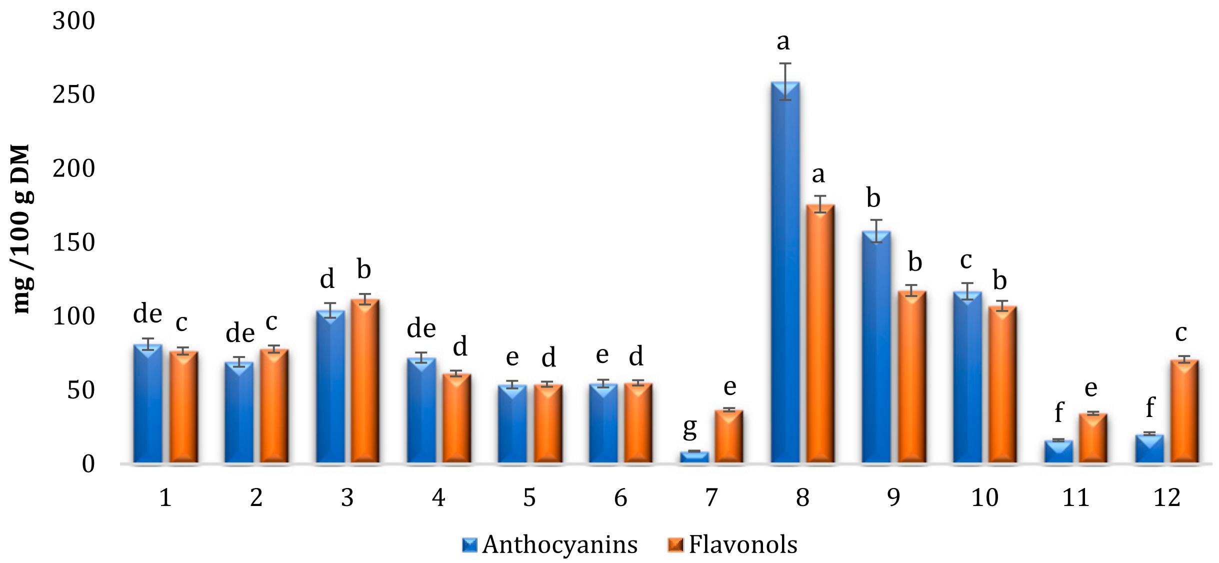

Figure 1 The content of anthocyanins and flavonols in the fruits of *Cornus mas* L. cultivars with an early ripening period: 1 – Radist; 2 – Olena; 3 – Ekzotychnyi; 4 – Nespodivanyi; 5 – Elegantnyi; 6 – Nikolka; 7 – Nizhnyi; 8 – Pervenets; 9 – Volodymyrskyi; 10 – Samofertylnyi; 11 – Alyosha; 12 – Koralovyi Marka (different superscripts in each column indicate the significant differences in the mean at p <0.05))

the NAS of Ukraine was in the range 477.1–850.0 mg% in the peel, 7.8–190.6 mg% the pulp (Klymenko, 2001). Studies of the content of anthocyanins in *Cornus mas* fruits were conducted in the Vlasina region (Serbia). The anthocyanin content was determined using spectrophotometric and high-performance liquid chromatography (HPLC) assays. The total anthocyanin content was 1383.2 mg/kg FW (Anđelković et al., 2015). This result is comparable to the results of our research.

New data for comparative analysis of different plant parts (leaf, flower and fruit) of *C. mas* are presented. Total phenolic content, flavonoid concentrations are analysed using *in vitro* standard spectrophotometric methods. Biological material of *C. mas* was collected from the region of Pčinja river gorge in south Serbia. Different solvents were used to extract flavonoids from the fruit: methanol, water, ethyl acetate, acetone, petroleum ether (Stankovic et al., 2014). Water extracts of *C. mas* fruits have indicators of 3.53 mg/g in terms of flavonoid content. These results have a higher result compared to our studies. *C. mas* fruit extracts with ethyl acetate show a very high result – 41.49 mg/g. The use of weakly polar solvents increases the completeness of the extraction of flavonoids from *C. mas* fruits.

As a result of our research, it was found that anthocyanin content in *C. mas* fruits with an early ripening period is in the range of 9–259 mg/100 g DM. The highest content of anthocyanins in fruits has the cv. Pervenets – 259 mg/100 g DM. Also, fruits of the cv. Volodymyrskyi have a fairly high anthocyanin content – 158 mg/100 g DM. The content of flavonols in the fruits of *C. mas* cultivars with an early ripening period is in the range of 34–176 mg/100 g DM. The highest content of flavonols in fruits also has the cv. Pervenets – 176 mg/100 g DM (Figure 1).

We conducted a correlation analysis between the content of anthocyanins and flavonols in the fruits of *C. mas* cultivars with an early ripening period. We found a very high correlation between the content of anthocyanins and flavonols in the fruits of *C. mas* plants with the early period of fruit maturation (r = 0.955).

The anthocyanin content in *C. mas* fruits with an intermediate ripening period is in the range of 10–166 mg/100 g DM. The highest content of anthocyanins in fruits has the cv. Mriia Shaidarovoi – 166 mg/100 g DM. Fruits of the cv. Vyshgorodskyi also has a fairly high anthocyanin content – 142 mg/100 g DM. The content of flavonols in the fruits of *C. mas* cultivars with an intermediate ripening period is in the range of 27–165 mg/100 g DM. The highest content of flavonols in fruits has the cv. Titus – 165 mg/100 g DM (Figure 2).

A correlation analysis between the content of anthocyanins and flavonols in the fruits of *C. mas* plants with an intermediate fruit ripening period showed a moderate correlation (r = 0.591).

The anthocyanin content in *Cornus mas* fruits with a late ripening period is in the range of 33–96 mg/100 g

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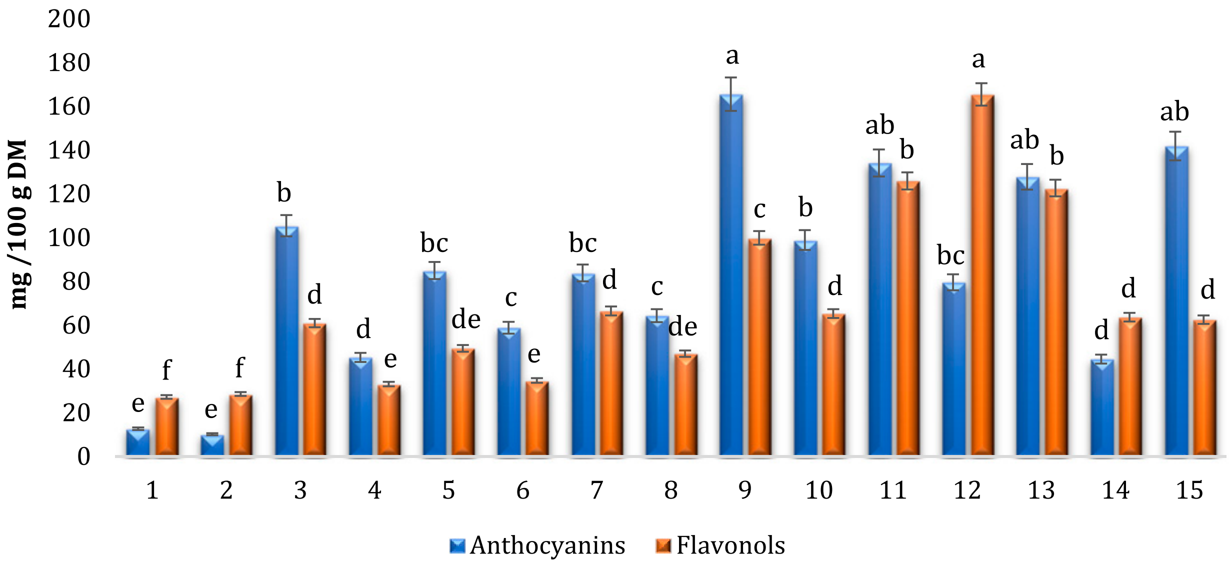

Figure 2 The content of anthocyanins and flavonols in the fruits of *Cornus mas* L. cultivars with an intermediate ripening period:
1 – Koralovyi; 2 – Yantarnyi; 3 – Medok; 4 – Priorskyi; 5 – Yevgeniia; 6 – Svitliachok; 7 – Vyshgorodskyi; 8 – Lukianivskyi; 9 – Mriia Shaidarovoi; 10 – Starokyivskyi; 11 – Yoliko; 12 – Titus; 13 – Oryginalnyi; 14 – Kostia; 15 – Vydubetskyi (different superscripts in each column indicate the significant differences in the mean at p <0.05))

DM. The highest content of anthocyanins in fruits has the cv. Sokolyne – 96 mg/100 g DM. The content of flavonols in the fruits of *C. mas* cultivars with a late ripening period is in the range of 36–88 mg/100 g DM. The highest content of flavonols in fruits also has the cv. Sokolyne – 88 mg/100 g DM (Figure 3).

Conducting a correlation analysis between the content of flavonols and anthocyanins for *C. mas* cultivars with a late ripening period showed a high level of correlation (r = 0.918).

We compared the average content of anthocyanins and flavonols in the fruits of *C. mas* cultivars with early, intermediate and late ripening. The diagram shows a decrease in the content of the studied compounds in cultivars with a late fruiting period. This may be due to a decrease in the length of daylight, which correlates with the quantitative content of anthocyanins and flavonols (Pan and Guo, 2016). *C. mas* cultivars with an early fruit ripening period were selected at the end of August, *C. mas* cultivars with an intermediate

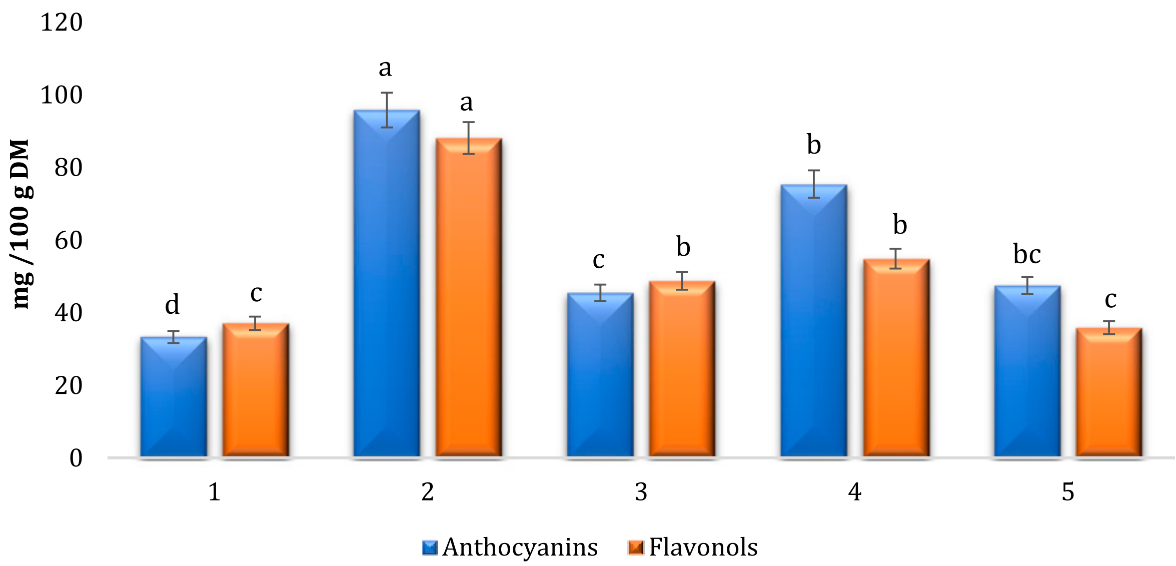

Figure 3 The content of anthocyanins and flavonols in the fruits of *Cornus mas* L. cultivars with a late ripening period:
1 – Semen; 2 – Sokolyne; 3 – Yuvileinyi Klymenko; 4 – Kozerig; 5 – Kolisnyk (different superscripts in each column indicate the significant differences in the mean at p <0.05))

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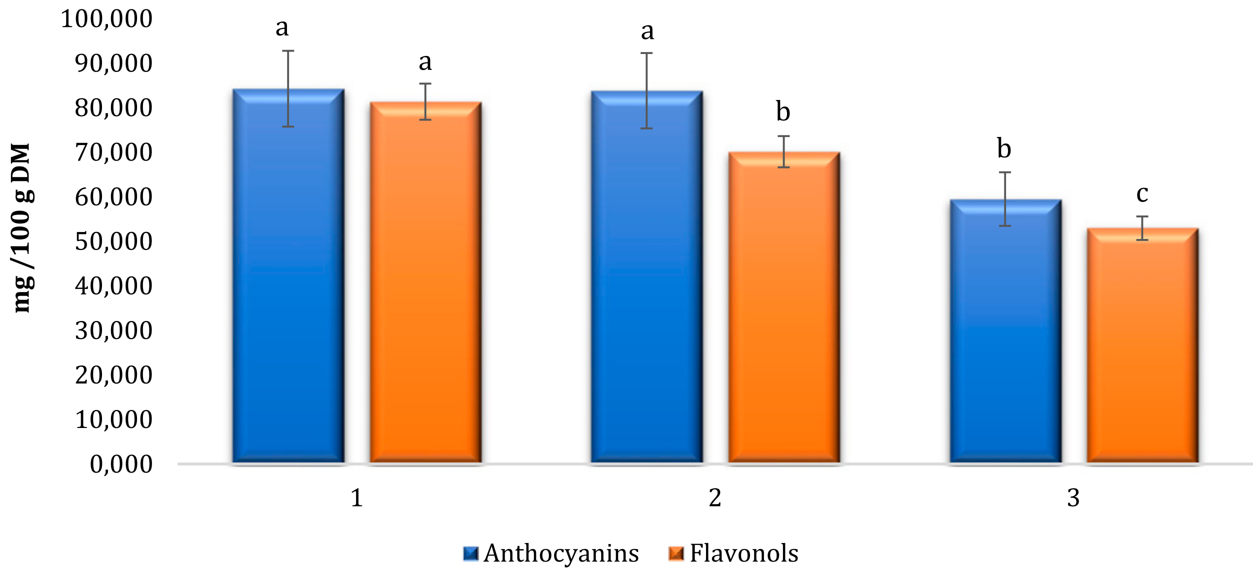

Figure 4 Comparison of the average anthocyanin and flavonol content in *Cornus mas* fruits with early, medium and late ripening periods:
1 – early fruiting; 2 – medium fruiting; 3 – late fruiting (different superscripts in each column indicate the significant differences in the mean at p <0.05))

fruit ripening period were selected at the beginning of September. In this case, the length of daylight has not changed significantly. *C. mas* cultivars with late fruit ripening were selected in early October. During this period, the daylight hours became much shorter. This difference is visible on the histogram (Figure 4).

It was found that the content of anthocyanins in the fruits of hybrid *C. mas* × *C. officinalis* significantly exceeds their content in the fruits of *C. officinalis* and cultivars of *C. mas* and amounts to 99–117 mg/100 g DM. The anthocyanin content in *Cornus officinalis* fruits is in the range of 52–93 mg/100 g DM, in comparison with the content of anthocyanins in the fruits of *C. mas* cultivars with a late ripening period in the range of 33–96 mg/100 g DM.

The content of flavonols in the fruits of hybrid *C. mas* × *C. officinalis* significantly exceeds their content in the fruits of *Cornus mas*, but in comparison with the content

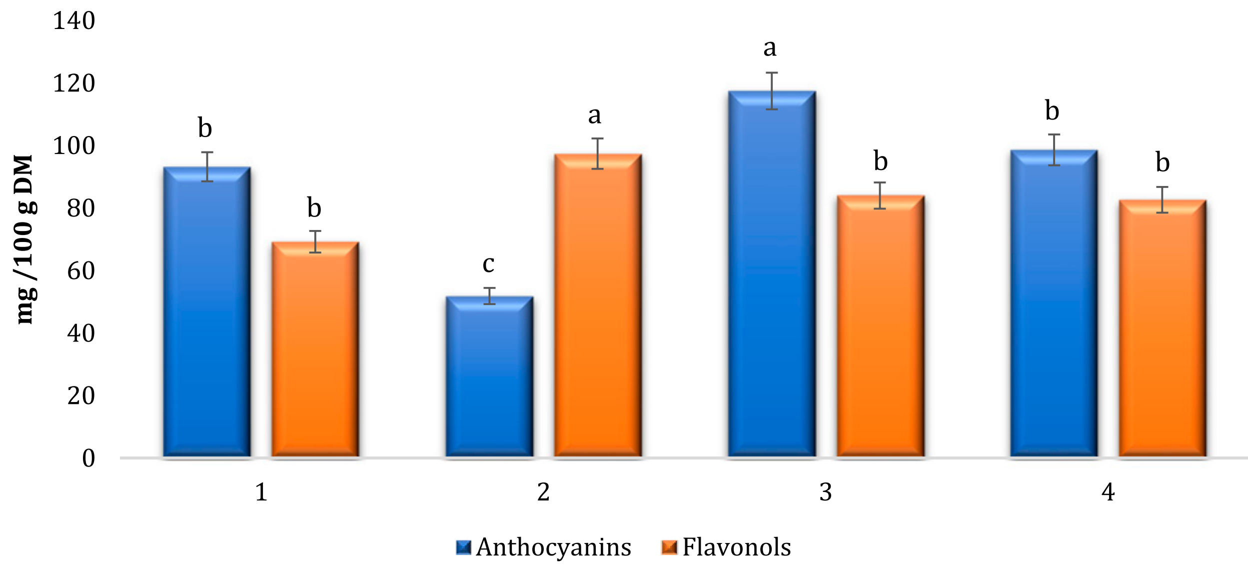

Figure 5 The content of anthocyanins and flavonols in the fruits of *Cornus officinalis* and *Cornus mas* × *Cornus officinalis* (cultivar Etude):
1 – *C. officinalis* (CO-01); 2 – *C. officinalis* (CO-02); 3 – cultivar Etude No 1; 4 – cultivar Etude No 2 (different superscripts in each column indicate the significant differences in the mean at p <0.05)

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of flavonols in the fruits of *C. officinalis* is within the same limits. The content of flavonols in the fruits of hybrid *C. mas* × *C. officinalis* is 83–84 mg/100 g DM, in the fruits of *C. officinalis* – 69–97 mg/100 g DM, in the fruits of *C. mas* cultivars with a late ripening period – 36–88 mg/100 g DM (Figure 5).

Conclusions

The amplitude of variability in the content of anthocyanins and flavonols in the fruits of *Cornus* spp. genotypes were studied. It is shown that the differences in the content of these compounds are determined by the genetic properties of the varieties and the ripening period of the fruits. Cultivars Pervenets, Volodymyrskyi, Mriia Shaidarovoi, Vydubetskyi, Titus, Sokolyne (*C. mas*), as well as hybrid *C. mas* × *C. officinalis* (cultivars Etude No. 1 and No. 2) have the highest content of these compounds. A correlation was found between the content of flavonols and anthocyanins in the fruits of *C. mas* cultivars. The correlation coefficient ranges from moderate (r = 0.591) in cultivars with an average fruit ripening period, too high (r = 0.955 and r = 0.918) in cultivars with an early and late fruit ripening period. A decrease in the average content of flavonols and anthocyanins in the fruits of *C. mas* cultivars with a late fruit ripening period was found. We assume that this may be due to a reduction in the length of daylight hours. High content of biologically active substances in *Cornus* fruits, including anthocyanins and flavonols, allows using raw materials as an active prophylactic and therapeutic agent.

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